

Amendments to the Specification:

Please replace the title with the amended title that follows. The amendment to the title is also shown in the accompanying Supplemental Application Data Sheet.

~~A Method of Purifying Factor VIII/vWF-Complex by Means of Cation Exchange~~
Chromatography and Methods of Purifying Same

Please replace the first paragraph on page 1, which was added in the preliminary Amendment filed February 27, 2004, with the following amended paragraph:

This application is a divisional of U.S. Patent Application No. 09/367,459, filed August 13, 1999, now U.S Patent No. 6,831,159, (which is incorporated herein by reference in its entirety), which is the U.S. National Phase of PCT/AT98/00043, filed February 27, 1998, which claims priority to Austrian Application No. A 338/97, filed February 27, 1997.

Please replace the two paragraphs at page 7, lines 3-15 with the following amended paragraphs:

Every known cation exchanger can be used for carrying out this method, cation exchangers having a sulfopropyl- or carboxymethyl-group conjugated carrier being preferred. The cation exchangers SP-Sepharose SP-SEPHAROSE® Fast Flow and CM-Sepharose CM-SEPHAROSE® Fast Flow (Pharmacia), Fractogel FRACTOGEL® EMD-SO3 and Fractogel FRACTOGEL® EMD COOH (Merck), Poros POROS® 10 SP and Poros POROS® 10 S (Perseptive Biosystems) and Toyopearl TOYOPEARL™ SP 550 C and Toyopearl TOYOPEARL™ CM-650 (M) (TosoHaas) have, e.g., proved to be well suitable.

A large-porous cation exchange gel having tentacle structure of the type of ~~Fractogel~~ FRACTOGEL® EMD-SO3 and Fractogel FRACTOGEL® EMD COOH (Merck) has proved particularly suitable for the recovery of purified vWF.

Please replace the paragraph starting on page 15, line 20 and bridging to page 16, line 18, with the following amended paragraph:

Usually the starting material is applied to the cation exchanger in a calcium-containing buffer. Immediately before application thereof, also a measure for inactivating potentially present viruses, such as human-pathogenic viruses which can be transmitted by blood, is conceivable. For this, a treatment with a virucidal detergent or with an organic solvent and/or detergent is preferred. A treatment with a detergent TRITON® Triton or Tween TWEEN® in the presence of TNBP (tri-(n-butyl)-phosphate) is, e.g., carried out according to EP 0 131 740. By a subsequent cation exchange chromatography the virucidal agent is effectively removed. If the adsorbed complex is washed, such washing preferably is effected with a washing buffer whose ionic strength is above that of the adsorption buffer, e.g. higher by 10-30%. For an elution of the factor VIII/vWF-complex, preferably the ionic strength is further increased. Elution of the factor VIII/vWF-complex is achieved by increasing the ionic strength, which preferably is increased by at least 50%, most preferred by at least 100%, as compared to the ionic strength of the starting solution. The elution buffer preferably contains sodium chloride. To formulate a pharmaceutical factor VIII/vWF-complex-preparation, usually diafiltration and sterile-filtration, as well as optionally a lyophilization, are effected.

Please replace the paragraph starting at page 18, line 20 and bridging to page 19, line 3 with the following amended paragraph:

A chromatographic column was filled with the cation exchanger Fractogel FRACTOGEL® EMD-SO3 and rinsed with buffer (30 mM glycine-NaCl-buffer). Subsequently, dissolved cryoprecipitate was filtered through the cation exchange column, and such proteins were obtained in the effluent which do not bind to the exchanger (Fraction 1). Unspecifically bound proteins were removed by rinsing the column with 0.3 M NaCl in buffer (Fraction 2).

Subsequently, FVIII/vWF-complex was eluted from the exchanger column by elution with 0.4 M and 0.5 M NaCl, respectively (Fraction 3 and Fraction 4, respectively).

Please replace the paragraph at page 21, lines 9-18 with the following amended paragraph:

Cryoprecipitate pre-treated in this manner was applied to a column of the cation exchanger Fractogel FRACTOGEL® EMD-TMAE. Non-bound proteins were obtained by rinsing the column with solution buffer (Fraction 1). This fraction contained 60% of the vWF activity, but merely 10% of the FVIII activity. By eluting the column with 400 mM NaCl (Fraction 2), FVIII/vWF-complex was subsequently obtained. Fraction 2 contained the remaining vWF activity and 70% of the FVIII activity, departing from the cryoprecipitate.

Please replace the paragraph on page 22, lines 9-18, which follow Table 2, with the following amended paragraph:

The FVIII/vWF-complex of Fraction 2 was diluted 4-fold with 20 mM glycine/NaCl-buffer, and subsequently applied to a cation exchange column of Fractogel FRACTOGEL® EMD-SO3. Non-binding proteins were obtained in Fraction 1. Weakly bound proteins were removed by rinsing the column with 200 mM NaCl (Fraction 2). Subsequently, it was eluted step-wise with 400 mM NaCl (Fraction 3) and 500 mM NaCl (Fraction 4). In each one of Fractions 3 and 4, 45% of the vWF-activity, and 55% or 40%, respectively, of the FVIII activities were found.

Please replace the paragraph starting on page 24, line 23 and bridging to page 25, line 3 with the following amended paragraph:

1000 ml of a cell culture supernatant containing recombinant rFVIII/rvWF-complex were applied onto a column filled with 20 ml of the cation exchanger Fractogel

FRACTOGEL® TSK-SO3. After having washed the column with buffer, pH 7.4, with 250 mM NaCl, the bound rFVIII/rvWF-complex was eluted by means of a buffer, pH 7.4, with 600 mM NaCl. In Table 4 the results of this column run are illustrated.

Please replace the two paragraphs at page 26, lines 14-22 with the following two amended paragraph:

Insoluble material, mainly fibrinogen, was separated. To inactivate possibly present pathogenic viruses, the clear solution was treated with 1% ~~Triton~~ TRITON® X100 detergent and 0.3% TNBP (tri-(n-butyl)-phosphate).

100 ml of the cation exchanger ~~Fractogel~~ FRACTOGEL® EMD-SO₃⁻-650 (M) from Merck Darmstadt (DE) were used for adsorption of the virus-inactivated FVIII, which previously had been equilibrated at pH 6.0 in an acetate-buffered NaCl solution having a conductivity of 10 mS/cm.

Please replace the paragraph at page 27, lines 2-3 with the following amended paragraph:

In this Example, the cation exchanger ~~Toyopearl~~ TOYOPEARL™ SP-550 C was used instead of ~~Fractogel~~ FRACTOGEL® EMD-SO₃⁻.